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MONOCLONAL ANTIBODY

Anti-MORC3 mAb

Code No.	Clone	Subclass	Quantity	Concentration
D238-3	17A9	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: MORC3 {microrchidia (MORC) family CW-type zinc finger 3, KIAA0136, ZCWCC3 and NXP-2}, is a member of the MORC protein family characterized by the conserved domains consisting of a GHL (Gyrase B, Hsp90 and MutL)-ATPase domain at the N-terminus, a zinc finger type CW domain containing conserved four cysteines and two tryptophans, a nuclear localization signal (NLS) and coiled-coil domains at the C-terminus. There are four MORC family proteins (MORC1, MORC2, MORC3, and MORC4) in human and five (Morc1, Morc2a, Morc2b, Morc3, and Morc4) in mice. MORC1 is expressed specifically in male germ cells, whereas MORC2 and MORC3 in human are ubiquitously expressed. The autosomal recessive mutation of *Morc1* in mice causes the arrest of spermatogenesis early in prophase I of meiosis. Takahashi *et al*. reported that MORC3 is involved in p53 activation and localization of conditionally localized p53 and constitutively localized Sp100 to promyelocytic leukemia (PML)-nuclear bodies (NBs) through its GHL-ATPase activity. MORC3 is localized in PML-nuclear bodies and entire nucleoplasm except for nucleoli.

SOURCE: This antibody was purified from hybridoma (clone 17A9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3X63-Ag8.653 with Balb/c mouse splenocyte immunized with recombinant human MORC3.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS:

Western blotting; 1-5 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested*
*It is reported that clone 17A9 can be used in this application in the reference number 1).
Immunohistochemistry; Not tested
Immunocytochemistry; 5 µg/mL
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HL60, U937, Jurkat, A431, HeLa, MCF7	WR19L, NIH/3T3, MEF	Rat1, PC12
Reactivity on WB	+	+	+

INTENDED USE:

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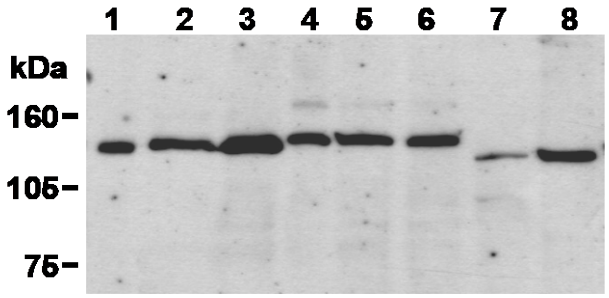
REFERENCES:

1) Ceribelli, A., *et al., Arthritis Res. Ther.* **14**, R97 (2012) [IP]
2) Mimura, Y., *et al., J. Cell Sci.* **123**, 2014-2024 (2010) [IF]
3) Takahashi, K., *et al., Mol. Biol. Cell* **18**, 1701-1709 (2007) [IF]
4) Inoue, N., *et al., Hum. Mol. Genet*. **8**, 1201-1207 (1999)



Western blot analysis of MORC3 expression in HL60 (1), U937 (2), A431 (3), WR19L (4) NIH/3T3 (5), MEF (6), Rat1 (7) and PC12 (8) using D238-3.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

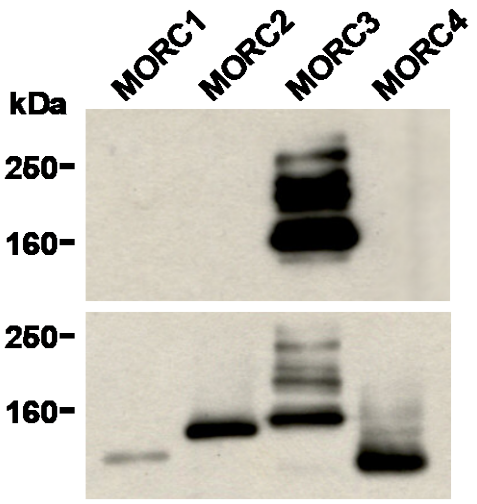
SDS-PAGE & Western Blotting

1) Wash the $1x10^7$ cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise

transfer procedure.

4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HL60, U937, A431, WR19L, NIH/3T3, MEF, Rat1, PC12)

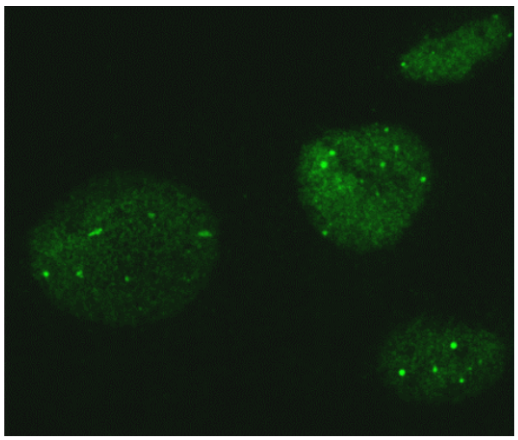


Western blot analysis of MORC3 expression in transfectant of MORC protein families using D238-3 (upper panel) or anti-DDDDK-tag (lower panel).
Expression plasmids of MORC protein families were kindly provided by Dr. Norimitsu Inoue. (Department of Molecular Genetics, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka 537-8511, Japan)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$ cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA)/PBS for 20 minutes at room temperature.
4) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
6) The glass slide was washed 3 times with PBS.
7) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 60 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add 30 µL of 1:600 Alexa Fluor® 488 Donkey Anti-Mouse IgG (Invitrogen; code no. A-21202) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add Permafluor™ aqueous mounting medium (MBL; code no. IM-0752) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Saos2)



Immunocytochemical detection of MORC3 on 4% PFA fixed Saos2 cells with D238-3.
This data was kindly provided by Dr. Norimitsu Inoue. (Department of Molecular Genetics, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka 537-8511, Japan)

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